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Biochemical variation and differentiation in the Brown hare *(Lepus europaeus)* of Central Europe

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Abstract

Genetic variation in 127 brown hares *(Lepus europaeus)* from populations in Poland, Hungary and Austria was investigated by horizontal starch gel electrophoresis. 25 isoenzyme systems were examined in kidney and liver tissues. For comparison, data on Czechoslovakian brown hares screened by Hartl (1987) were also included in this study. From 39 loci scorable, 10 were polymorphic. Values of polymorphism (mean $\overline{P} = 0.163$) and expected average heterozygosity (mean $\overline{H} = 0.048$) were found to be similar to the corresponding mean values in mammals. Genetic differentiation between populations, however, is remarkably low, even over large geographic distances. These results are discussed with respect to genetic variability and differentiation in other lagomorphs, such as the wild rabbit and the pika.

Introduction

In contrast to other groups of mammals such as rodents, insectivores, seals and artiodactyles the amount and distribution of genetic variability in wild living populations of lagomorphs is only poorly known. Population genetic data obtained by electrophoretic multilocus investigations are available only for rabbits *(Oryctolagus cuniculus)* from Australia (Richardson 1980, 1981; Richardson et al. 1980; Daly 1981) and for pikas (*Ochotona princeps*– Glover et al. 1977; Tolliver et al. 1985) and cottontails (*Sylvilagus floridanus* – Scribner et al. 1983; van den Bussche et al. 1987) from North America.

Although the brown hare *(Lepus europaeus)* has been subjected to various biochemical-systematic investigations to evaluate the genetic distance from some close relatives (e.g. Schneider and Leipoldt 1983; Bonhomme et al. 1986; Hartl 1987), population genetic data are still lacking in this species. To get a first large scale impression on the amount and distribution of genetic variation in the brown hare we scored individuals from different sampling sites in Poland, Austria, Czechoslovakia and Hungary. The results are discussed with respect to the distribution of this species in Central Europe and to the population genetic data in other lagomorphs already studied, such as the wild rabbit and the pika.

Material and methods

A total of 127 brown hares was investigated in this study. The distribution of sampling sites in Poland, Austria and Hungary is shown in the map (Fig. 1). Tissue samples were collected either by local hunters or by members of the institutes mentioned in the list of authors' addresses. Liver and kidney were frozen immediately after death of the specimens and were stored either in liquid nitrogen or in a freezing box at −20 °C until electrophoresis. Preparation of tissue extracts, electrophoretic and staining procedures as well as the interpretation of electrophoretic band-patterns were performed according to routine methods (Hartl and Höger 1986; Hartl 1987).

The following 25 isoenzyme systems were screened (abbreviation and E.C. number are given in parentheses): α-glycerophosphate dehydrogenase (GDC, E.C. 1.1.1.8), sorbitol dehydrogenase (SDH, E.C. 1.1.1.14), lactate dehydrogenase (LDH, E.C. 1.1.1.27), malate dehydrogenase (MOR, E.C. 1.1.1.37), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), 6-phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44), glucose dehydrogenase (GDH, E.C. 1.1.1.47), glucose-6-phosphate dehy-

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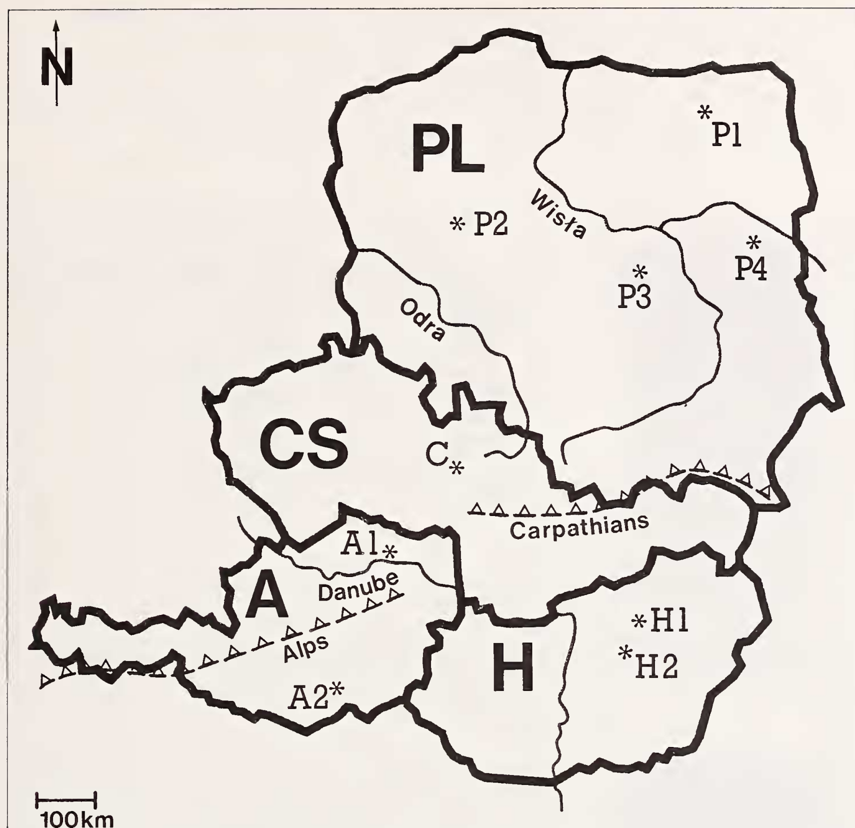


Fig. 1. Distribution of sampling sites in Poland (P1–P4), Hungary (H1, H2), Austria (A1, A2) and the origin of founder individuals of a brown hare breed at Litovel in Czechoslovakia (C)

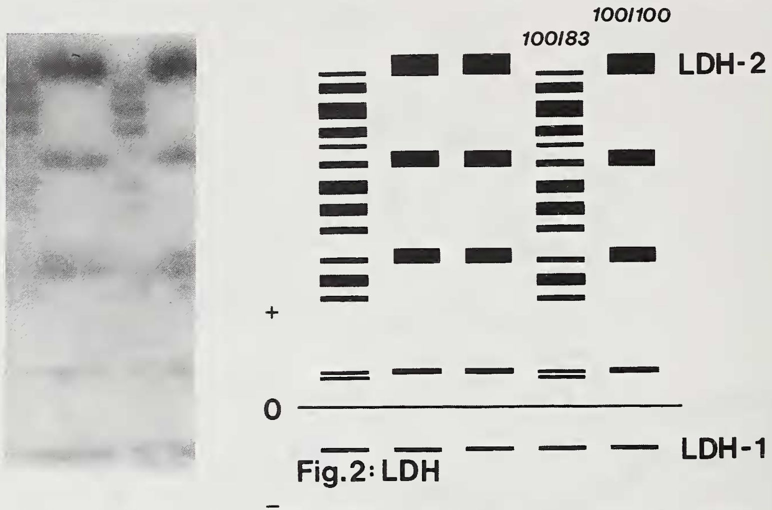
drogenase (GPD, E.C. 1.1.1.49), xanthine dehydrogenase (XDH, E.C. 1.2.3.2), glutamate dehydrogenase (GLUD, E.C. 1.4.1.3), catalase (CE, E.C. 1.11.1.6), superoxide dismutase (SOD, E.C. 1.15.1.1), glutamate oxaloacetate transaminase (GOT, E.C. 2.6.1.1), hexokinase (HK, E.C. 2.7.1.1), creatine kinase (CK, E.C. 2.7.3.2), adenylate kinase (AK, E.C. 2.7.4.3), phosphaglucomutase (PGM, E.C. 2.7.5.1), esterases (ES, E.C. 3.1.1.1), acid phosphatases (ACP, E.C. 3.1.3.2), peptidases (PEP, E.C. 3.4.11), aminoacylase-1 (ACY-1, E.C. 3.5.1.14), adenosine deaminase (ADA, E.C. 3.5.4.4), fumarate hydratase (FH, E.C. 4.2.1.2), mannosephosphate isomerase (MPI, E.C. 5.3.1.8) and glucosephosphate isomerase (GPI, E.C. 5.3.1.9).

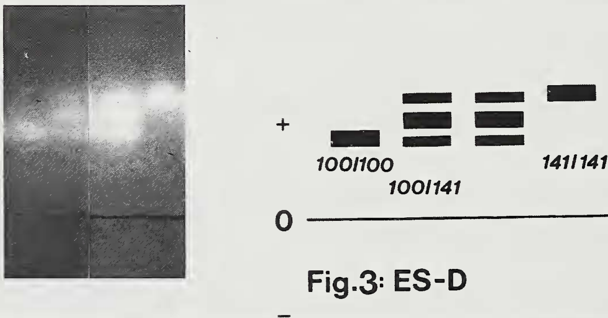
For comparison also the data on 25 brown hares from a breed at our institute (Hartl 1987) were included in the present study. ES-D was screened additionally in the same specimens, to make the data totally comparable. All these individuals are the offspring of a parent generation (24 animals) obtained from a breeding station at Litovel (ČSSR). This breed originates from wild living brown hares captured at the site shown in the map (Fig. 1, ČSSR).

Results

Screening of 25 enzyme systems representing 39 presumptive structural loci revealed polymorphism in 9 isoenzymes: LDH-2, MOR-2, IDH-2, PGD, ES-I, ES-D, PEP-2, ACY-1 and MPI. Additionally HK-2 is polymorphic in the Czechoslovakian hares screened by Hartl (1987). As an example, zymograms of the polymorphic isoenzymes

LDH-2 and ES-D as well as the genetic interpretation of band-patterns are shown in Figs. 2 and 3. Allele frequencies at the polymorphic enzyme loci are given in Table 1, values of polymorphism, heterozygosity and average heterozygosity are given in Table 2. The following isoenzymes were monomorphic in all specimens studied: GDC, SDH, LDH-1, MOR-1, IDH-1, GDH-2, GPD, XDH, GLUD, CE, SOD-1, -2, GOT-1, -2, HK-1, -3, CK-2, AK-2, -3, PGM-1, -2, ACP-1, -2, -3, PEP-1, ADA-1, FH, GPI-1 and -2.





Figs. 2–3. Electrophoretic and diagrammatic representation of some polymorphic isozenzymes in the brown hare

The total amount of genetic variation was analysed using NEI's (1975) measures of gene diversity. The average diversity among populations (D_{ST}) was 0.0028 and accounted for approximately 5.5 per cent (G_{ST} = 0.055) of the total gene diversity (H_T = 0.051).

Genetic relationships between populations were estimated using a number of different distance and cluster algorithms. The distance measures used were those compiled by NEI et al. (1983) and ROGERS (1986). Dendrograms were constructed using the UPGMA as described by NEI (1975), WAGNER networks according to FARRIS (1972). Furthermore, FITCH-MARGOLIASH and CAVALLI-SFORZA – EDWARDS trees were constructed using the PHYLIP package of FELSENSTEIN (1985). NEI's (1978) distances corrected for small sample sizes are given in Table 3. Fig. 4 shows a dendrogram which was constructed by means of

Table 1. **Allele frequencies at the polymorphic enzyme loci in the brown hare populations studied (99 % criterion)**
n = sample size of individuals

Population		H1	H2	P1	P2	P3	P4	C	A1	A2
n		17–30	25	16	5	10	12	19–25	12	17
Enzyme locus	allele	allele frequencies								
Ldh-2	*100*	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.91
	83	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.09
Mor-2	*100*	0.98	0.98	0.94	0.90	0.90	1.0	1.0	1.0	1.0
	121	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	79	0.0	0.02	0.06	0.10	0.10	0.0	0.0	0.0	0.0
Idh-2	*100*	0.98	0.96	1.0	1.0	1.0	1.0	0.98	1.0	0.94
	130	0.0	0.02	0.0	0.0	0.0	0.0	0.02	0.0	0.0
	83	0.02	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.06
Pgd	*100*	0.92	0.96	0.97	0.90	1.0	1.0	0.98	1.0	0.97
	170	0.07	0.02	0.0	0.10	0.0	0.0	0.0	0.0	0.0
	129	0.0	0.0	0.03	0.0	0.0	0.0	0.02	0.0	0.03
	117	0.01	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hk-2	*100*	1.0	1.0	1.0	1.0	1.0	1.0	0.95	1.0	1.0
	67	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.0	0.0
Es-1	*−100*	0.47	0.58	0.41	0.80	0.55	0.63	0.72	0.83	0.77
	−108	0.09	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	−75	0.32	0.42	0.59	0.20	0.45	0.33	0.28	0.17	0.23
	−42	0.12	0.0	0.0	0.0	0.0	0.04	0.0	0.0	0.0
Es-d	*100*	0.75	0.86	0.81	0.80	0.85	0.96	0.92	0.75	0.82
	141	0.25	0.14	0.19	0.20	0.15	0.04	0.08	0.25	0.18
Pep-2	*100*	0.77	0.72	0.81	0.90	0.85	0.63	0.74	0.77	0.71
	104	0.23	0.28	0.19	0.10	0.15	0.37	0.26	0.23	0.29
Acy-1	*100*	0.33	0.34	0.28	0.10	0.10	0.38	0.14	0.42	0.33
	81	0.40	0.42	0.66	0.60	0.70	0.37	0.74	0.33	0.38
	66	0.27	0.24	0.06	0.30	0.20	0.25	0.12	0.25	0.29
Mpi	*100*	0.98	1.0	0.81	0.90	1.0	1.0	1.0	0.96	1.0
	126	0.02	0.0	0.19	0.10	0.0	0.0	0.0	0.04	0.0

At each polymorphic isoenzyme locus the most common allele in population A 1 was designated arbitrarily *"100"*, variant alleles in the same or in other populations according to their relative mobility

the UPGMA using Nei's (1972) standard genetic distances. A comparison of the dendrograms based on different clustering algorithms and genetic dissimilarity measures displays the following relationships among the populations studied: The "eastern cluster" (H1, H2, P4), the "western cluster" (A1, A2) and the "northern cluster" (P2, P3, C) have been found in almost all cases, whereas the subclustering in the clusters consisting of three members changed frequently. To test the influence of sample size and the composition of genetic loci chosen the well known bootstrap and jackknife methods have been applied to automatically produce a range of comparable dendrograms. Constructed was a majority rule consensus tree (PHYLIP, Felsenstein 1985) for a hundred Nei (1972) distance/UPGMA trees, where the allele frequencies were simulated using multinomial distribution with the allele frequencies observed. The same procedure was followed, where for every tree distances were recalculated after randomly omitting 25 % of the genetic loci investigated. Whereas the jackknife consensus tree confirms the result by using all genetic loci

Table 2. **Genetic variability in the brown hare populations studied; the values are calculated over 39 loci**

Population	H1	H2	P1	P2	P3	P4	C	A1	A2
n	17–30	25	16	5	10	12	25	12	17
Enzyme locus	expected heterozygosity (H) observed heterozygosity (H_o)								
Ldh-2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.161
	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.177
Mor-2	0.033	0.039	0.118	0.180	0.180	0.0	0.0	0.0	0.0
	0.033	0.040	0.125	0.200	0.200	0.0	0.0	0.0	0.0
Idh-2	0.033	0.078	0.0	0.0	0.0	0.0	0.039	0.0	0.111
	0.033	0.080	0.0	0.0	0.0	0.0	0.040	0.0	0.118
Pgd	0.154	0.078	0.060	0.180	0.0	0.0	0.039	0.0	0.056
	0.167	0.080	0.062	0.200	0.0	0.0	0.040	0.0	0.059
Hk-2	0.0	0.0	0.0	0.0	0.0	0.0	0.100	0.0	0.0
	0.0	0.0	0.0	0.0	0.0	0.0	0.105	0.0	0.0
Es-I	0.652	0.487	0.482	0.320	0.495	0.497	0.403	0.278	0.360
	0.529	0.360	0.437	0.400	0.300	0.500	0.240	0.333	0.353
Es-d	0.375	0.241	0.305	0.320	0.255	0.080	0.147	0.375	0.290
	0.367	0.280	0.250	0.400	0.300	0.083	0.160	0.167	0.235
Pep-2	0.360	0.403	0.305	0.180	0.255	0.469	0.384	0.351	0.415
	0.471	0.400	0.250	0.200	0.300	0.583	0.440	0.273	0.353
Acy-1	0.658	0.650	0.487	0.540	0.460	0.656	0.418	0.653	0.663
	0.700	0.640	0.500	0.800	0.500	0.583	0.440	0.417	0.765
Mpi	0.033	0.0	0.304	0.180	0.0	0.0	0.0	0.081	0.0
	0.033	0.0	0.187	0.200	0.0	0.0	0.0	0.083	0.0
$\overline{P}$	0.205	0.180	0.180	0.180	0.128	0.103	0.180	0.128	0.180
$\overline{H}$	0.060	0.051	0.053	0.049	0.042	0.044	0.039	0.045	0.053
$\overline{H}_o$	0.060	0.048	0.046	0.062	0.041	0.045	0.038	0.033	0.053

n = sample size of individuals, $\overline{P}$ = proportion of polymorphic loci, $\overline{H}$ = expected average heterozygosity, $\overline{H}_o$ = observed average heterozygosity

Table 3. **Genetic identities – above diagonal – and genetic distances – below diagonal – calculated according to Nei (1978)**

	H1	H2	P1	P2	P3	P4	C	A1	A2
H1	–	1.0000	0.9974	0.9991	0.9983	0.9991	0.9965	0.9988	0.9992
H2	0.0000	–	0.9981	0.9992	0.9991	1.0008	0.9981	0.9991	1.0001
P1	0.0026	0.0019	–	0.9976	0.9997	0.9961	0.9967	0.9940	0.9948
P2	0.0009	0.0008	0.0024	–	1.0014	0.9979	1.0005	1.0004	1.0002
P3	0.0017	0.0009	0.0003	−0.0014	–	0.9975	0.9999	0.9961	0.9974
P4	0.0009	−0.0008	0.0039	0.0021	0.0025	–	0.9980	0.9992	1.0004
C	0.0035	0.0019	0.0033	−0.0005	0.0001	0.0020	–	0.9967	0.9980
A1	0.0012	0.0009	0.0060	−0.0004	0.0039	0.0008	0.0033	–	1.0009
A2	0.0008	0.0000	0.0052	−0.0003	0.0026	−0.0004	0.0020	−0.0009	–

(Fig. 4), telling us that no single locus has an overwhelming influence on the topology of the tree, the bootstrap consensus tree exhibits a striking difference with respect to the position of P2 due to the small sample size (n=5) available from this population.

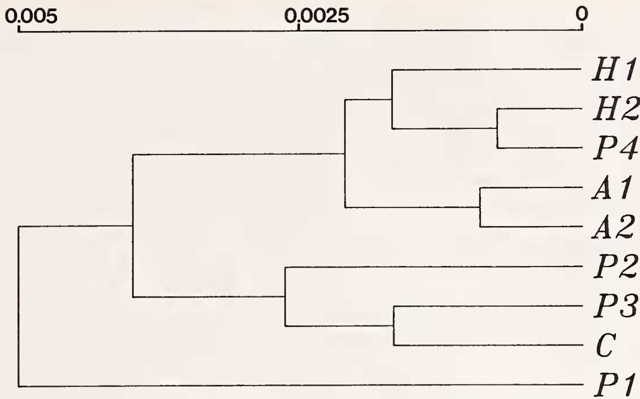


Fig. 4. Dendrogram constructed by means of the UPGMA using NEI's (1972) standard genetic distance

Discussion

The values of polymorphism (mean $\overline{P}$ = 0.163, sd. = 0.034) and expected average heterozygosity (mean $\overline{H}$ = 0.048, sd. = 0.007) detected in the present study on the brown hare are somewhat lower than those found by BONHOMME et al. (1986) in samples from Spain, possibly due to the larger number of rapidly evolving proteins examined by these authors (transferrin, several esterases). They are, however, closely similar to the mean values in mammals calculated over 184 species by NEVO et al. (1984). If genetic variation in brown hare populations (Table 2) is compared with population genetic data on other lagomorphs, quite similar values of polymorphism and heterozygosity have been found in a small sample of wild rabbits by HARTL (1987; $\overline{P}$ = 0.132; $\overline{H}$ = 0.042), where exactly the same set of genetic loci was examined. Similar results have also been obtained in an investigation of 26 loci in approximately 200 wild rabbits from England, France, Tasmania and mainland Australia ($\overline{P}$ = up to 0.192; $\overline{H}$ = 0.060) by RICHARDSON et al. (1980). Whereas the extent of polymorphism and heterozygosity in populations of the brown hare and the wild rabbit seems to be rather equal, remarkably lower values of genetic variation, especially of average heterozygosity, were detected in a study on electrophoretic variation at 26 loci in 165 pikas from Colorado (USA; mean $\overline{P}$ = 0.115, sd. = 0.044; mean $\overline{H}$ = 0.005, sd. = 0.002; GLOVER et al. 1977). These results were confirmed by a later study on some more pika populations from Colorado carried out by TOLLIVER et al. (1985), where the low heterozygosity in the pika is explained as the result of founder effect and genetic drift occurring in the small isolated populations of this species during postglacial shifts in its range. However, since in a population from Montana (USA) a $\overline{P}$-value of 0.154 and a $\overline{H}$-value of 0.046 were detected by GLOVER et al. (1977), apart from the situation in Colorado, also the pika may have an extent of genetic variation similar to that in the brown hare and the wild rabbit.

Thus, as far as average values of polymorphism and heterozygosity are concerned, the ecological differences between the three species of lagomorphs do not seem to be reflected by substantial differences in the extent of biochemical genetic variation within populations. However, when the proportion of polymorphic loci is calculated for the whole species (by summing up all polymorphisms found in the different local populations instead of taking an average) the pika with 37 % (calculated from TOLLIVER et al. 1985) shows much the same value as the brown hare (38.5 %; present study), but the rabbit is different with

19.2 % (calculated from RICHARDSON et al. 1980). Furthermore, the proportion of polymorphisms shared by all populations studied is 40 % in the brown hare but only 10 % in the pika (TOLLIVER et al. 1985). These differences between species can be explained in various ways. The lower extent of polymorphism in the rabbit may either be an artefact because of different enzyme loci examined (other, possibly additional, polymorphisms were detected in Austrian rabbits by HARTL 1987) or the result of its history of distribution (see e. g. BARRETT and RICHARDSON 1986). The set of enzymes examined by GLOVER et al. (1977) and TOLLIVER et al. (1985) is rather similar to that investigated in the present study and the low percentage of polymorphisms shared by pika populations may be generally the result of its existence in so called „terrestrial islands" (GLOVER et al. 1977), which are of course more susceptible to losses of alleles by genetic drift than a more panmictic network of populations, occurring for instance in the European brown hare.

The data obtained in the present study suggest, that there is very low genetic differentiation not only between populations inhabiting continuous agricultural regions but also between populations occurring on different sides of broad rivers (Wisła, Danube) or mountain chains (Alps, Carpathians). In spite of the rather high genetic diversity within populations of the brown hare the genetic diversity between populations is comparatively low (G_{ST} = 5.5 %) and also genetic distances (calculated according to NEI 1978) are negligible in most cases. Since genetic distances are mainly influenced by the distribution of allele frequencies at the highly polymorphic loci, the high degree of genetic homogeneity between the populations studied may be caused to a certain extent by selective forces acting on these loci (see e. g. RICHARDSON 1980; PEMBERTON et al. 1988).

This hypothesis is supported by the data on Czechoslovakian animals. Although they were subjected to breeding for several generations, they are not very different in their allele frequencies from the other populations studied. Furthermore, their position in the various dendrograms is fitting quite well to the expectations derived from the geographical site of their origin. However, the selection hypothesis needs to be tested in more detailed investigations and the genetic similarity within the study area may simply be the result of a high amount of gene flow, which is possibly prevented by broad rivers, leading to the rather stable main clusters shown in Fig. 4.

Acknowledgements

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Zusammenfassung

Biochemische Variation und Differenzierung beim mitteleuropäischen Feldhasen (Lepus europaeus)

Die genetische Variabilität von 127 Feldhasen *(Lepus europaeus)* aus Populationen in Polen, Ungarn und Österreich wurde mit Hilfe der horizontalen Stärkegelelektrophorese ermittelt. Dazu wurden 25 Isoenzymsysteme aus Nieren- und Lebergewebsextrakten untersucht. Zum Vergleich mit Feldhasen aus der Tschechoslowakei wurden in der vorliegenden Arbeit auch von HARTL (1987) erhobene Daten herangezogen. Von 39 auswertbaren Genloci zeigten 10 einen Polymorphismus. Die gefundenen Polymorphie- (durchschnittl. $\bar{P}$ = 0.163) und Heterozygotieraten (durchschnittl. $\bar{H}$ = 0.048) liegen nahe dem generell für Säugetiere errechneten Mittelwert. Die genetische Differenzierung zwischen Populationen ist jedoch, auch über größere geographische Entfernungen hinweg, sehr gering. Diese Befunde werden unter Bezugnahme auf die genetische Variabilität und Differenzierung bei anderen Lagomorphen, wie etwa dem Wildkaninchen und dem Pfeifhasen, diskutiert.

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